Analysis of genetic diversity for wild and captive green peafowl populations by random amplified polymorphic DNA technique

KE Ya-yong[1], CHANG Hong[2], ZHANG Guo-ping[2]

[1] *Wild Fauna and Flora Protection and Management Office, Guangdong Forestry Bureau, Guangzhou 510173, P. R. China*

[2] *School of Life Science, Zhongshan University, Guangzhou 510275, P. R. China*

Abstract: The genetic diversity of the populations for 14 wild green peafowls (*Pavo muticus*) and 18 captive green peafowls was investigated by using the technology of random amplified polymorphic DNA (RAPD). Totally 161 and 166 amplified bands were obtained by using 23 arbitrary primers to amplify the genomic DNA of wild and captive green peafowls respectively. The results showed that the average relative genetic distance of the wild and captive green peafowls populations was 0.0555 and 0.1355, respectively, and difference of the average relative genetic distances between the two populations was 0.1635. The Shannon diversity index for the wild and captive green peafowl populations was 0.4348 and 1.0163, respectively, which means that there exists significant difference in genetic diversity between the two populations, and the genetic diversity of wild green peafowl was low. The two populations originated from two different families according to analysis by the UPGMA method. This research can provide the theoretical basis for supervising genealogies management of peafowl populations.

Keywords: Green peafowl; *Pavo muticus*; Genomic DNA; Random amplified polymorphic DNA (RAPD)

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Introduction

There are two peafowl species in Asia, ie. green peafowl (*Pavo muticus*) and blue peafowl (*Pavo cristatus*). The green peafowl is only found in western, southern and central Yunnan Province of China (Wen *et al.* 1995). It is estimated that the number of green peafowl in China is 800-1100. As the population of green peafowl is small, this study was only undertaken to assess its genetic diversity and genetic diversity trend. Some green peafowl have been reared in captivity for a period of time. This offered to an opportunity to compare the change of genetic diversity between the captive and wild populations. Recent studies on green peafowl mostly focused on ecology and foraging skill (Hong *et al.* 2002)., This study, is carried out on the genetic diversity of the wild and captive green peafowl populations by using the random amplified polymorphic DNA (RAPD) method, aimed at providing the theoretical basis on supervising genealogies management for peafowl populations

Materials and methods

Blood samples of peafowl

Blood samples for wild green peafowl were collected from the individuals marked from No.1-14 (7 males, 7 females) in the rescue center of Yunnan Province and those for domestic green peafowl were collected from the individuals marked from No.1-18 (9 males, 9 females) at the Yingjili peafowl farm of Huadu district in Guangzhou City. All the blood samples have been marked relatively.

DNA extraction

Genomic DNA was extracted by using the high salt solution method (Jian *et al.* 1996). Uncoagulated blood samples of 300 uL were rinsed for three times with 1.2-mL cell cracking solution [10-mmol Tris-Cl (pH=7.5), 10-mmol NaCl, 10-mmol $MgCl_2$]. The leucocyte karyon was cracked with 500-mL leucocyte karyon cracking solution [10-mmol Tris-Cl (pH=8.0), 400-mmol NaCl, 2-mmol EDTA]. The precipitate was digested in the solution of 700-mL leucocyte karyon cracking with 10-uL protein K (10 mg/mL) and 100-uL SDS (10%) at 50 °C. The digested solution was purified by extracting once with chloroform and isopentanol [1:1(vol/vol)]. Samples were finally concentrated by ethanol precipitation, and dissolved in TE (Tris-HCl of 10 mmol/L, EDTA of 1 mmol/L, pH 8.0).

RAPD primers

The 23 primers which had stable amplified products from 41 arbitrary primers (Sangon Company) were selected for their sequences (Table 1).

Polymerase chain reaction

Amplification was performed in 25 uL of a solution containing Tris-HCl of 10 mmol/L (pH=8.0), KCl of 50 mmol/L,

Biography: KE Ya-yong (1975-), male, Postgraduate in Wild Fauna and Flora Protection and Management Office, Guangdong Forestry Bureau, Guangzhou 510173, P. R. China (E-mail: nature218@yahoo.com.cn).

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NP of 0.08%, MgCl of 2.0 mmol/L and dNTP of 0.2mmol/L, while the solution also contains 11 pmol of each primer, genomic DNA (25ng), and 1.0 unit of Thermus aquaticus polymerase (Sangon companty). The mixture above was covered with 20-uL mineral oil. The polymerase chain reaction consisted of pre-denaturation for 200 seconds at 95 °C, then denaturation for 30 seconds at 94 °C, anneal for 60 seconds at 36 °C, and extension for 120 seconds at 72 °C. This cycle was repeated 40 times (PERKIN GeneAmp PCRSsystem 2400). Electrophoresis of 25-uL amplified mixture was done in a 1.4%-agarose gel with 0.05% ethidium bromide. DNA markers were supplied by Lambda DNA /Hind III and EcoR I Markers.

Table 1. Sequence of arbitrary primers and total number of bands

Primers	Sequence(5'→3')	Total number of bands		Primers	Sequence(5'→3')	Total number of bands	
		wild	captive			wild	captive
S8	G T C C A C A C G G	11	12	S505	G A C C T A G T G G	3	5
S77	T T C C C C C C A G	5	5	S506	G T C T A C G G C A	7	7
S80	A C T T C G C C A C	8	10	S507	A C T G G C C T G A	6	6
S84	A G C G T G T C T G	10	7	S509	T G A G C A C G A G	5	5
S91	T G C C C G T C G T	9	9	S511	G T A G C C G T C T	7	8
S94	G G A T G A C A C C	8	8	S512	A C A G G T G C G T	9	10
S104	G G A A G T C G C C	3	4	S513	G G A C G A C A A G	7	7
S159	A C G G C G T A T G	9	7	S515	G G A C A A C G A G	7	6
S164	C C G C C T A G T C	7	8	S516	C T C T G C G C G T	6	7
S501	T G C G G G T C C T	6	6	S517	C C G T A C G T A G	4	4
S503	A C A C A G A G G G	9	9	S520	A C G G C A A G G A	8	8
S504	C C C G T A G C A C	7	8				

Product analysis

Bands were scored throughout the comparison of the bands and DNA markers. The coefficient of band sharing (index of similarity) was calculated in the following formula.

$$F=2N_{xy}/(N_x+N_y) \quad (1)$$

where, N_x and N_y present the numbers of bands of individuals x and y respectively, and N_{xy} is the number of bands shared by x and y.

The relative genetic distance (D) was calculated in the following formula.

$$D=1-F \quad (2)$$

where, F is the index of similarity (Nei *et al.* 1979).

Based on the relative genetic distance, the pedigree of the wild and captive populations was constructed with the Unweighted Pair-group Method and Arithmetical Average (UPGMA) method through PHYLIP program (ver.3.5c).

The genetic diversity (H_o) was calculated by Shannon diversity index in the following formula.

$$H_o = -\Sigma \pi_i \ln \pi_i \quad (3)$$

where, π_i is the frequency of a band that presents in the population of number i (King *et al.* 1989). For n populations, the Shannon diversity index was calculated in the following formula.

$$H_{pop}=(\Sigma H_0)/n \quad (4)$$

where, H_{pop} is the inner diversity index of n populations; ΣH_0 is the summation of the diversity index of n populations; *n is-* the number of population.

The genetic diversity of total populations (H_{sp}) by calculating the phenotype frequency (π) was calculated in the following formula.

$$H_{sp} = -\Sigma \pi \ln \pi \quad (5)$$

The proportion of genetic variation (V) between the wild and captive populations in the total genetic variation was calculated in the following formula.

$$V= (H_{sp}-H_{pop})/ H_{sp} \quad (6)$$

where, (H_{sp}- H_{pop}) means the diversity index between the wild and captive populations.

Based on the H_0 (genetic diversity) of the wild and captive populations, we carried out the *t*-Test through the software of SPSS for Window (release 9.0), then we can check whether the H_0s is different remarkably.

Results and discussion

The amplified bands were clearly obtained when all 23 primers were used to amplify the 32 genomic DNA of green peafowl. The 54 diversity segments of a total of 161 amplified segments were obtained when 21 primers were used to amplify genomic DNA of wild green peafowl. In a total of 166 amplified segments obtained, there were 99 diversity segments when all 23 primers were used to amplify genomic DNA captive green peafowl (Fig 1).

Analysis of genetic diversity

The relative genetic distance of individuals was calculated based on the amplified products (Table 2). The average relative genetic distance was only 0.0555 for wild green peafowl population, and was 0.1355 for captive green peafowl population. At the same time, the average relative genetic distance between the wild and captive populations was 0.1635, which was larger than that in ei-

ther of the two populations. The Shannon diversity index also indicated that the genetic diversity of captive green peafowl population (1.0163) was much larger than that of wild green peafowl population (0.4348). Furthermore, most of the genetic variation of the two populations appeared in inner of the population. A notable difference was found between wild and captive populations. These results show that the genetic diversity of the wild green peafowl population in China is poor. It is necessary to protect the wild animals and avoid excessively inbreeding of the captive animals as soon as possible. One of the effective measures may be to build a forest corridor to promote gene communication between isolated populations. It is also necessary to study the trend of genetic diversity in wild and captive populations.

Genealogy and breed management

The wild and captive populations' genealogy was constructed by using UPGMA method based on the data of their relative genetic distance. The genealogy showed that for the wild green peafowl population, the thirteenth female individual and fourteenth male individual which have a close relationship belong to one large group, and other twelve individuals remain with another large group. The thirteenth female individual and fourteenth male individual can be used to crossbreed with others twelve individuals to improve the genetic diversity of their offspring. The captive population could be also classified into two large groups, one group consisting of individual No. 3, 1, 2, 4, 5, 6, 7 and 8; the other group consisting of individual No. 9 and 10, 16, 17 and 18, 11 and 12, 15, 13 and 14. For long-term genetic management of the wild and captive populations, it is recommended that individuals of the two groups in wild and captive populations are allowed to crossbreed each other in each population in avoiding further inbreeding.

Table 2. Matrix of relative genetic distances among green peafowl individuals

Individual No.	Individual No.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	—	.04	.05	.04	.05	.07	.05	.06	.07	.06	.03	.07	.08	.06				
2	.08	—	.03	.03	.06	.08	.06	.06	.05	.04	.05	.05	.08	.05				
3	.06	.08	—	.01	.04	.07	.05	.05	.04	.03	.05	.04	.07	.07				
4	.08	.10	.04	—	.03	.06	.04	.04	.04	.03	.04	.04	.06	.06				
5	.06	.08	.09	.05	—	.06	.05	.04	.04	.05	.04	.05	.05	.07				
6	.07	.08	.05	.05	.05	—	.06	.07	.07	.06	.06	.09	.09	.09				
7	.07	.07	.07	.07	.06	.05	—	.05	.05	.05	.04	.06	.07	.07				
8	.05	.09	.07	.08	.06	.06	.05	—	.04	.05	.04	.05	.05	.08				
9	.19	.19	.18	.16	.17	.18	.16	.18	—	.03	.05	.06	.08	.06				
10	.21	.22	.21	.19	.19	.19	.18	.18	.12	—	.05	.05	.08	.05				
11	.21	.21	.21	.19	.19	.19	.18	.19	.08	.11	—	.07	.06	.06				
12	.17	.17	.18	.16	.15	.17	.16	.16	.09	.11	.09	—	.06	.07				
13	.18	.17	.18	.16	.16	.17	.14	.17	.09	.13	.08	.09	—	.08				
14	.19	.18	.19	.17	.17	.18	.17	.17	.09	.11	.09	.07	.07	—				
15	.20	.19	.20	.17	.18	.19	.16	.19	.12	.16	.11	.10	.09	.09	—			
16	.20	.21	.19	.17	.18	.19	.17	.19	.13	.11	.11	.10	.11	.10	.10	—		
17	.18	.18	.20	.17	.16	.18	.15	.16	.08	.09	.09	.08	.09	.06	.10	.10	—	
18	.20	.18	.20	.19	.19	.19	.15	.18	.12	.13	.10	.11	.09	.09	.07	.09	.05	—

Notes: Data above the diagonal line represent the relative genetic distance of wild green peafowl, and those under the diagonal line represent the relative genetic distance of the captive green peafowl.

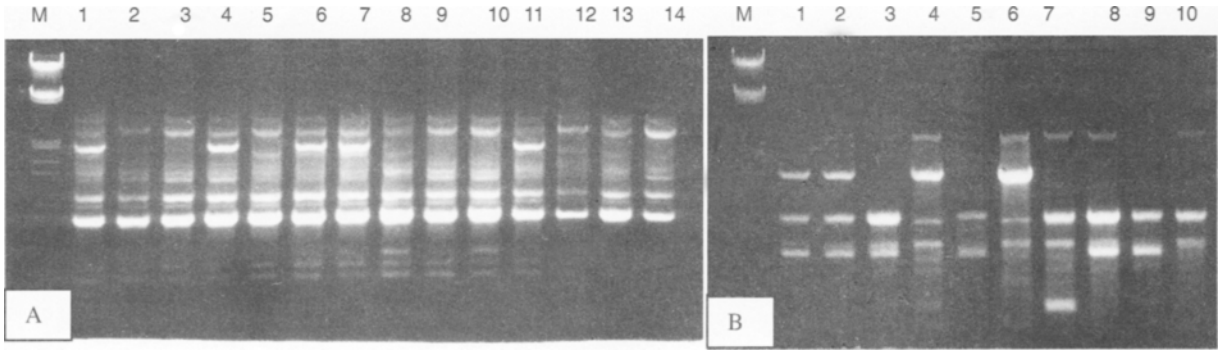

Fig. 1 The PCR products of wild and captive green peafowls

Note: A: PCR product of primer S511 in wild green peafowl population; B: PCR product of primer S511 in captive green peafowl population. M: Lambda DNA /Hind III + EcoR I DNA Markers. 1-14: 14 individuals No. of wild green peafowl; 1-10: 10 individuals No. of captive green peafowl.

Table 3. Partitioning of the genetic diversity within and between wild and captive green peafowl population

Primers	H_o		Hpop	Hsp	Hpop/Hsp	V
	Wild	Captive				
S8	0.7091	2.3575	1.5333	2.0767	0.7383	0.2617
S77	0.2009	0.0540	0.1275	0.1220	1.0446	-0.0446
S80	0.4574	1.6690	1.0632	1.9266	0.5518	0.4482
S84	0.6647	1.2688	0.9668	2.0262	0.4771	0.5229
S91	1.4190	1.8973	1.6581	1.5481	1.0711	-0.0711
S94	0.6737	1.0819	0.8778	0.9770	0.8984	0.1016
S104	0.1885	0.6608	0.4246	0.6270	0.6773	0.3227
S159	0.3261	0.2350	0.2806	0.6484	0.4328	0.5672
S164	0.3631	1.7605	1.0618	1.4341	0.7404	0.2596
S501	0.0000	0.5385	0.2692	0.3217	0.8369	0.1631
S503	0.2840	0.3579	0.3209	0.3357	0.9560	0.0440
S504	0.1376	1.5259	0.8317	1.1833	0.7029	0.2971
S505	0.0000	0.7578	0.3789	1.4728	0.2573	0.7427
S506	0.2780	0.5513	0.4146	0.4493	0.9228	0.0772
S507	0.6457	1.1159	0.8808	1.4207	0.6200	0.3800
S509	0.5054	0.3265	0.4159	0.4167	0.9983	0.0017
S511	0.8527	1.7917	1.3222	1.5850	0.8342	0.1658
S512	0.2840	0.8657	0.5749	0.7599	0.7565	0.2435
S513	0.7097	1.1245	0.9171	0.9706	0.9449	0.0551
S515	0.3216	0.7827	0.5521	0.9187	0.6010	0.3990
S516	0.3904	0.7334	0.5619	0.8976	0.6260	0.3740
S517	0.0000	0.1519	0.0760	0.0892	0.8515	0.1485
S520	0.5884	1.7675	1.1779	1.7534	0.6718	0.3282
Average	1.0163	0.4348	0.7256	1.0418	0.7483	0.2517

Notes: H_o----the genetic diversity; Hpop-----the inner diversity index of different populations; Hsp-----the genetic diversity of total populations; Hpop/Hsp----the ratio of inner population genetic variation to the total genetic variation; V----the ratio of genetic variation between the wild and captive green peafowl populations to the total genetic variation.

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